Probiotic fermented foods and health promotion

1

A.C. Ouwehand[1], *H. Röytiö*[2]
[1]DuPont, Kantvik, Finland; [2]University of Turku, Turku, Finland

1.1 Introduction

Food fermentation has, throughout much of human history, been the most common way of preserving perishable foods, thereby maintaining and in some cases even improving the nutritional value of these foods. Genesis 18:8 refers to how Abraham serves curds and milk to his guests. Not surprisingly, some of these fermented foods were perceived to be inherently healthy. The mechanism behind this preservation was not clarified until 1857, when Louis Pasteur identified "lactic yeast" as the source of lactic acid fermentation. A first "scientific" promotion of fermented food specifically as a health product came in the early 1900s with Ilya Metchnikoff, who advertised yogurt, fermented with the Bulgarian bacillus, and insisted it would contribute to longevity (Metchnikoff, 1907). In the 1930s, Minoru Shirota specifically isolated a health-promoting microbe and introduced the oldest still-existing probiotic food, Yakult.

Probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). Maintaining viability imposes some technological requirements on the manufacturing of the probiotic food product; the minimal counts should be guaranteed until the end of shelf life. The required level of these counts is likely to depend on the probiotic strain and the intended health benefit. As a rule of thumb, a minimum of 10^9 colony-forming units (CFU)/consumption is used (Forssten, Sindelar, & Ouwehand, 2011). A correct approach would be to use a minimum dose according to that used in studies documenting the given health benefit.

Although probiotics are widely consumed as dietary supplements, the focus of the present chapter is on fermented probiotic foods. Most commercially available probiotics belong to the genera *Bifidobacterium* and *Lactobacillus*; strains from other genera are being marketed as well, but these rarely find application in fermented foods and will thus not be discussed here.

1.2 Probiotic fermented foods and health promotion

Probiotics can be included in many different foods, fermented and unfermented. The food matrix is known to have an important role in the stability of probiotics (Forssten et al., 2011). Manufacturers take this into account when developing such foods. However, which role the matrix plays in efficacy is less well understood. For some strains

Advances in Fermented Foods and Beverages. http://dx.doi.org/10.1016/B978-1-78242-015-6.00001-3
Copyright © 2015 Elsevier Ltd. All rights reserved.

it does not seem to play a role, while it does for others. This topic requires further investigation and is not discussed here owing to lack of information. It is important to realise that diet is likely to be a bigger cause of variation than the matrix of one or another probiotic food (Kelly, Colgan, & Frank, 2012).

1.2.1 Different types of fermented probiotic foods

Numerous fermented foods exist, but not all of these food classes can be linked with the probiotic concept, such as alcoholic beverages and fermented meats, or foods in which the fermentations merely fulfil a technological function in the processing of the food, such as in coffee, tea and cocoa.

1.2.2 Fermented dairy foods/beverages

Fermented dairy foods are the most widely used carriers of probiotics in Western societies, in particular yogurt and yogurt-type drink products. This may have historic reasons as mentioned above, but it has also practical reasons. Most commercially available probiotics belong to the genera *Bifidobacterium* and *Lactobacillus*. Members of these genera tend to grow well in milk, and it may even be their most common habitat.

In fermented probiotic dairy products, probiotics are usually accompanied by starter cultures such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and/or *Staphylococcus thermophilus*. There are two main reasons for the inclusion of starter cultures in a probiotic product. The first is technological: starter cultures provide structure and flavour to the product. In addition, starter cultures support functionality; some probiotics do not grow well as a pure culture in milk and grow better in symbiosis with a starter culture.

Besides fresh fermented dairy products, probiotics can be included in nonfermented milk such as the so-called "sweet acidophilus milk". The milk is not sweet in the sense of sweet taste, but is referred to as such because it is not sour (Mcdonough, Hitchins, Wong, Wells, & Bodwell, 1987).

Furthermore, probiotics can be included in cheese. Despite the long ripening and shelf life of cheese, probiotic counts appear to be stable in cheese for months. By optimizing fermentation techniques, it is feasible to produce a good-quality cheese with high probiotic counts so that a standard portion of cheese (15 g) provides a dose of at least 10^9 CFU (Ibrahim et al., 2010).

1.2.3 Fermented soy foods/beverages

In Asian societies, fermented soy foods are common. They are, however, not typically used to function as a delivery matrix for probiotics. Probiotic soy products most commonly designed to resemble dairy products are called "soy yogurts". Lactic fermentation of "soy milk" may improve the bioavailability of isoflavones, improve mineral availability and increase the level of B vitamins (Rekha & Vijayalakshmi, 2010). Fermentation has the additional advantage of reducing the bean flavour of soy foods. In general, soy yogurts aim at providing a vegetarian/vegan alternative to regular (probiotic) yogurt and are not developed specifically as carriers for probiotics. Nevertheless,

the stability of probiotics in a fermented soy matrix is usually good and is very similar to that in yogurt.

1.2.4 Fermented plant foods/beverages

Lactic fermented plant foods are common in Asian, African and East European societies. These are fermented vegetables such as sauerkraut and kimchi, which are mainly based on spontaneous fermentations dictated by the storage conditions and ingredients used for this fermentation (Jung et al., 2011).

Lactic fermented cereals are common, such as in sourdough, although obviously subsequent processing (baking) will not allow survival of microbes. Lactic fermentation of cereals otherwise contributes to improved flavour and reduces phytic acid activity, thereby improving biological availability of minerals such as iron. Lactic acid bacteria involved in the fermentation may also produce vitamins, in particular B vitamins (Nout, 2009). Traditionally, fermented foods of vegetable or cereal origin have not been used as carriers for probiotics. However, it cannot be excluded that the microbes involved in these fermentations have a direct influence on health, similar to a probiotic. Specifically designed foods are successfully marketed, usually on the basis of fermented cereals, and have been studied as probiotic carriers. Such products have only sporadically been used as carriers of probiotics (Molin, 2001). The stability of selected probiotics in fermented cereal-based products has been documented to be good.

1.2.5 Other carriers of probiotics

Probiotics have been included successfully in ice cream. Relevant to this book is the production of ice cream based on frozen yogurt, which basically follows the same manufacturing as probiotic yogurt (Davidson, Duncan, Hackney, Eigel, & Boling, 2000). But probiotics can also be included in the ice cream base or in a chocolate coating of the ice cream.

As mentioned earlier, supplements are probably the most common format for probiotic consumption, in addition to dairy products. Furthermore, probiotics have been included in fruit juices, which is particularly challenging because of the low pH (<4) and the presence of various natural antimicrobial components in fruits (such as benzoate and anthocyans). Probiotics have also been included in chocolate (Vriens, 2009), providing extremely long shelf lives of up to 2 years – not so much to produce probiotic chocolate bars, but to provide a probiotic-carrying chocolate coating to various foods. These applications fall outside the focus of this chapter, but indicate that formats for probiotics exist.

1.3 Health benefits deriving from the consumption of probiotics

As discussed above, the role that the food matrix plays in the efficacy of probiotics, if any, has been insufficiently investigated. Many studies have been performed with

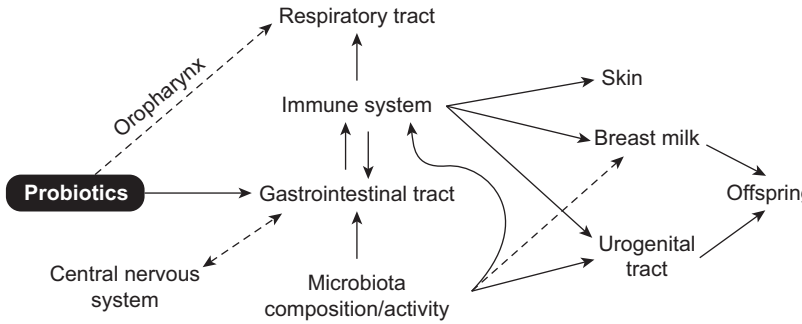

Figure 1.1 Schematic representation of how consumed probiotics may exert their benefits in the intestine and beyond.

probiotics as supplements because it is easier to produce a placebo and the logistics are easier than for a fresh fermented dairy product. We therefore present below the results of probiotic consumption, regardless of the matrix in which it was consumed. Although probiotics are consumed, they can have health benefits beyond the gastrointestinal tract. Potential mechanisms for these extra-intestinal effects are depicted in Figure 1.1.

1.4 Gastrointestinal health

Given that probiotics are consumed, it is not surprising that the prime target for probiotic use has traditionally been various diarrhoeas, with intestinal health as the main benefit.

1.4.1 Modulating microbiota composition

With the advances in molecular biological techniques, such as metagenome sequencing, 16S rDNA pyrosequencing, and quantitative real-time polymerase chain reaction, an unprecedented understanding of the composition and genetic diversity of the intestinal microbiota has been possible. International research consortia have sequenced intestinal microbiomes of an increasing number of subjects. At the same time, metabolomics provides us with an ever deeper insight into the metabolic capacity of the intestinal microbiota. Notwithstanding these advances, it is still not possible to define a "healthy" or "normal" microbiota (Qin et al., 2010).

One of the early targets of probiotics has been the improvement of the composition of the intestinal microbiota. From the above, it is obvious that criteria for "improving" intestinal microbiota composition are at present not clearly defined. Nevertheless, some options exist for improving intestinal microbiota. Keeping under control potential pathogens such as *Clostridium difficile* and *Helicobacter pylori*, which can be present in low numbers in the microbiota of asymptomatic healthy carriers, may be beneficial (see following text). Furthermore, although the composition of a "healthy" or "normal" microbiota is not known, it is assumed that the microbiota a

healthy subject carries is appropriate for that particular host. It is therefore reasonable to assume that allowing a disturbed microbiota (as a consequence of whatever stress) to return to "normal" quicker or reduce the aberrance from "normal" is a health benefit. However, increasing levels of faecal bifidobacteria, hitherto one of the traditional targets, is not sufficient. The proof that increasing *Bifidobacterium* numbers is a health benefit per se is lacking.

1.4.2 Infectious diarrhoea

Infectious diarrhoeas can have many different aetiologies, viz., viral, bacterial, protozoal or chemical. The various causes also make it a challenging target. In children, the effect of probiotics on the duration of rotavirus diarrhoea has been well documented for various strains (Van Niel, Feudtner, Garrison, & Christakis, 2002). Despite this and other documented benefits of probiotics for children, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) does not encourage the widespread use of probiotics in infant nutrition (Braegger et al., 2011). In adults, travellers' diarrhoea is relatively common, but probiotics have shown inconsistent results (Mcfarland, 2007; Ritchie & Romanuk, 2012). This inconsistency is most likely attributable to the heterogeneity of diarrhoeal causes.

1.4.3 Antibiotic-associated diarrhoea

Antibiotics have provided great medical progress in the treatment of bacterial infectious diseases. However, because of their antimicrobial nature, one of their side effects is a disturbance of the intestinal microbiota. This antibiotic-related disturbance was recognised even in the early days of therapeutic use of antibiotics around 1950, and, as a response, the term "probiotic" was probably first introduced by Kollath in 1953 and Vergio in 1954 (Holzapfel & Schillinger, 2002). This kind of disturbance, or "dysbiosis", may lead to intestinal complaints and, in the worst cases, to antibiotic-associated diarrhoea (AAD). AAD is caused by various pathogens, most notably *C. difficile*, followed by *Staphylococcus aureus* and *Clostridium perfringens*. However, these three pathogens together explain, at most, only half of the reported cases of AAD. The cause of the remaining AAD cases is largely unknown. Many probiotic strains and combinations of probiotic strains have been documented to reduce the incidence and/or duration of AAD. This is among one of the best documented health benefits of probiotics (Hempel et al., 2012). Probiotics do not replace the microbes that are reduced by antibiotic therapy, but support the microbiota in maintaining or returning more quickly to its original composition (Engelbrektson et al., 2009). Probiotics are highly successful in the prevention, shortening and/or relief of AAD, whereby one case of AAD can be prevented for every eight patients treated with probiotics (Avadhani & Miley, 2011).

1.4.4 Necrotising enterocolitis

Premature infants, and in particular very low birth weight infants, are at risk of developing necrotising enterocolitis (NEC). The mortality of NEC is substantial. Probiotics

provide one of the few preventive treatment options. While many probiotics on their own do not provide a significant reduction in NEC incidence, meta-analyses clearly show a reduced incidence of NEC and reduced mortality (Wang, Dong, & Zhu, 2012). Although the aetiology of NEC is poorly understood, it is likely to have an infectious component. By competitive exclusion, probiotics may prevent the establishment of potentially pathogenic microbes. Although preterm infants are a population at risk for infection, administering probiotics has not been reported to lead to adverse events; sepsis has neither been reported to be reduced by probiotic therapy nor been observed to be increased. As almost all tested probiotics have been observed to reduce NEC risk, it is tempting to argue that the often suggested strain specificity of health effects is not valid for NEC and that in this case there is a health benefit of probiotics in general.

1.4.5 Helicobacter pylori *infection*

Helicobacter pylori can be a resident of the gastric mucosa. It is the causative agent of gastric ulcer and is thought to cause gastric cancer. Most probiotics have been selected to survive gastric conditions and many produce antimicrobial substances; probiotics could therefore be effective against *H. pylori* infection. However, this appears not to be the case. Nevertheless, probiotics can play a valuable role in *H. pylori* eradication, which has serious side effects. In analogy with the effects of probiotics on AAD, probiotics have been observed to reduce the side effects of *H. pylori* eradication therapy, thereby improving compliance and also otherwise improving the success rate (Wang, Gao, & Fang, 2013).

The question is whether we should strive for the eradication of *H. pylori*. Carriage or prior exposure to the organism has been found to be associated with reduced risk for allergy and obesity. It is possible that *H. pylori* should only be kept under control, and probiotics could play an important role here.

1.4.6 Intestinal transit

Slow colonic transit is associated with constipation, though not all constipation is caused by slow transit: transit may be normal, but obstruction may cause constipation. Constipation correlates well with hard stools, as measured with the Bristol Stool Scale (Lewis & Heaton, 1997). There are several ways to determine intestinal transit, i.e. scintigraphy, radio-opaque pellets and remote recording capsules. Dyes have also been used, but these are inaccurate as their excretion is strongly correlated with defecation frequency. Slow colonic transit is thought to increase the risk of, among others, colorectal cancer and diverticulitis. Sufficient fluid and fibre intake are recommended but are not always adequate. Shortening of intestinal transit should not lead to diarrhoea. Selected probiotic strains such as *Bifidobacterium animalis* subsp. *lactis* HN019 and *B. animalis* subsp. *lactis* DN-173 010 have been documented to normalise slow intestinal transit (Ibrahim & Ouwehand, 2011; Waller et al., 2011). The mechanism by which probiotics are able to shorten colonic transit remains to be established, although short chain fatty acid production may be one of the contributing factors.

1.4.7 Irritable bowel syndrome

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders in industrialised countries, affecting an estimated 2–20% of the population (Rey & Talley, 2009). IBS is usually diagnosed according to the so-called Rome III criteria, which comprise prolonged and recurrent abdominal pain or discomfort along with disturbances in bowel movements (diarrhoea and/or constipation). Along with this, patients usually experience gastrointestinal symptoms such as bloating, abdominal distension and flatulence. Although the exact mechanisms behind IBS are still unclear, visceral sensory and motor disturbances, as well as increased gut permeability and inflammation, may play an important role. IBS patients appear to have an altered microbiota in comparison to healthy subjects; whether this is the cause or the effect of IBS is, however, currently unknown. Probiotics such as *Bifidobacterium infantis* 35624 have shown beneficial effects on symptoms of IBS (Brenner, Moeller, Chey, & Schoenfeld, 2009). However, the beneficial effects are often symptom-specific and seem to depend on the specific strain, dose and duration (Clarke, Cryan, Dinan, & Quigley, 2012). The proposed mechanisms behind the efficacy of probiotics include decreasing pain radiation, as has been shown for *Lactobacillus acidophilus* NCFM (Rousseaux et al., 2007), and colonisation of pathogenic/gas-producing bacteria in the intestine and modulating the host immunity (Clarke et al., 2012).

1.4.8 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is, as the name suggests, a collection of chronic inflammatory conditions of the intestine, such as ulcerative colitis (UC), referring to inflammation of and restricted to the colon; Crohn disease, being an inflammation that can be anywhere from mouth to anus; and pouchitis, and inflammation of an ileoanal pouch that has been constructed after severe UC. The aetiology of the disease is poorly understood but the microbiota is clearly involved, as germ-free animals prone to IBD do not develop the disease until they are colonised with a microbial population. One of the microbiota components involved in IBD has been found to be a lack or low level of *Faecalibacterium prausnitzii* (Sokol et al., 2009). The disease can be brought into remission with anti-inflammatory drugs, but tends to flare up after some time (Hedin, Whelan, & Lindsay, 2007). Probiotics have not been successful in inducing remission, but they have been found to prolong remission. In particular, a multi-strain probiotic product, VSL#3 (consisting of *Bifidobacterium longum*, *B. infantis*, *Bifidobacterium breve*, *L. acidophilus*, *Lactobacillus casei*, *L. delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum* and *S. thermophilus*), has been shown to be successful in the treatment of pouchitis (Mimura et al., 2004).

1.5 Immune health

The human gastrointestinal microbiota, as well as microbiota of other mucosal body surfaces, consists of a large number of bacterial species that are living in symbiosis

with the host and are able to cross-talk with the intestinal and adaptive immune system. It has become obvious that different microbes are able to influence the development and function of the immune system, and the balance between the so-called beneficial versus potentially harmful microbes has a pivotal role in either the maintenance or disruption of the inflammatory control. Therefore, the protective and immunomodulatory role of probiotic microbes has been investigated in several aspects of the immune system function and different infectious diseases (Purchiaroni et al., 2013).

1.5.1 Respiratory tract infections

The common cold or upper respiratory tract infection (URTI) is a frequently occurring disease in all age groups. URTI are usually viral infections and are the reason for the largest proportion of school and work absenteeism. None of the current interventions (vitamins, remedies and antibiotics) are very effective in preventing colds. The effect of probiotics is still less well studied, and reviews and meta-analyses on the topic show a slightly positive effect but variable results, mainly owing to the diverse strains and doses used and different age groups tested (Hao, Lu, Dong, Huang, & Wu, 2011; Kang, Kim, Hwang, & Ji, 2013). Thus, also in this application, the effects seem to be strain dependent. In a double-blind, placebo-controlled study with young children (3–5 years of age) attending daycare, *L. acidophilus* NCFM alone or in combination with *B. animalis* subsp. *lactis* Bi-07 was effective in reducing fever, rhinorrhoea and cough duration and the incidence of antibiotic prescription, as well as the number of missed daycare days. The supplementation continued for 6 months over the winter period (Leyer, Li, Mubasher, Reifer, & Ouwehand, 2009). In another study with older children (aged between 8 and 13 years), a two-strain probiotic product with *L. acidophilus* and *Bifidobacterium bifidum* consumed twice a day for 3 months was effective in reducing the risk of fever, cough, rhinorrhoea or school absence in comparison to placebo (Rerksuppaphol & Rerksuppaphol, 2012). Also, college students living on campus (aged between 18 and 24) gained benefits from consuming *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis animalis* subsp. Bb-12 during the spring semester; the duration of URTI was reduced by 2 days in the probiotic group and they also recorded lower disease severity scores in comparison to placebo group. Moreover, the number of missed school days attributable to illness was less in the group receiving probiotics compared to the placebo group (Smith, Rigassio-Radler, Denmark, Haley, & Touger-Decker, 2013). Furthermore, in highly active adult athletes at higher risk to develop URTI because of increased endurance-based physical activity, the use of *L. casei* Shirota for 16 weeks reduced the frequency of URTI episodes, although the severity and duration of the illness were not affected (Gleeson, Bishop, Oliveira, & Tauler, 2011). Although the results from studies on adult populations are mixed, it is tempting to conclude that probiotics would be an effective method to reduce the incidence of this very common disease in children and in adults that causes a huge economic burden owing to school and work absenteeism. The precise mechanism of protective effects remains to be elucidated, but it has been speculated that probiotics are able to compete against pathogens, enhance the barrier function in respiratory epithelia, and stimulate the cellular

immunity in airways to confer their protective effects against URTI (Ahanchian, Jones, Chen, & Sly, 2012).

1.5.2 Allergies, asthma and atopic dermatitis

An ever-increasing number of children in developed countries suffer from allergies, asthma and atopic dermatitis (AD), which are regarded as a failure in the development of a balanced immune response. The highly increased prevalence of these diseases indicates that environmental factors play a significant role in the disease development, as the escalation has been too fast for a shift in the genetic constitution to occur. The lack of microbial exposure early in life attributable to changed living conditions and diet are thought to lead to a dysbiosis in the microbiota composition and skew the immune response against common environmental antigens, leading to loss of oral tolerance and development of clinical symptoms (Fujimura, Slusher, Cabana, & Lynch, 2010). Reduced bacterial diversity in the infant's gut has been associated with an increased risk of allergic sensitisation and allergic rhinitis (Bisgaard et al., 2011), as well as atopic dermatitis (Nylund et al., 2013). Therefore, the protective role of probiotics has been investigated in these diseases, and probiotic supplementation early in life and also prenatally (used by the mother during pregnancy) seems to have a role in preventing AD and IgE-associated AD (Doege et al., 2012; Pelucchi et al., 2012; Toh, Anzela, Tang, & Licciardi, 2012). In initial randomised human studies using *L. rhamnosus* GG, it was shown that with probiotic supplementation in the mothers' diet before delivery and in infants after birth, a 50% reduction in AD prevalence in a high-risk population was achieved (Kalliomäki et al., 2001), while the protective effect persisted at least up to 7 years of age (Kalliomäki et al., 2007). Very similar results have also been demonstrated for another *L. rhamnosus* strain (HN001) (Wickens et al., 2008; Wickens et al., 2012). The key protective mechanism of *L. rhamnosus* against eczema may be related to its ability to promote the growth of other beneficial microbial species in the gut, resulting in a more stable and functionally redundant gastrointestinal community of the infant (Cox et al., 2010). In allergic diseases, the evidence of protective effects of probiotics is more limited (Toh et al., 2012), although some recent data point to reduced severity of seasonal pollen allergies in children and in adults. *L. acidophilus* NCFM and *B. animalis* subsp. *lactis* Bl-04 alleviated the symptoms of allergic rhinitis in children during the birch pollen season, and this effect was shown to derive from the prevention of pollen-induced infiltration of eosinophilic cells into the nasal mucosa during probiotic supplementation (Ouwehand et al., 2009). In adults, *B. animalis* subsp. *lactis* NCC2818 has also been shown to impact the function of blood lymphocytes and reduce concentrations of proinflammatory cytokines, and thus mitigate allergic symptoms during grass pollen season (Singh et al., 2013). The anti-inflammatory effect may derive from the same mechanisms as in eczema: the probiotic microbes improve the balance of the normal microbial composition and thus impact the function of the immune cells and the clinical symptoms of allergies. Intriguingly, the microbiota composition of allergic individuals has indeed been shown to fluctuate during the allergy seasons, and probiotic

intervention reduces this fluctuation (Odamaki et al., 2007; Ouwehand et al., 2009). This seems to hold true also in food allergies, where altered microbiota composition is seen between allergic and healthy individuals (Canani & Di Costanzo, 2013; Noval Rivas et al., 2013), although no evidence on the protective effects of probiotics exists to date. However, many studies have failed to demonstrate the protective effects of probiotics against allergies and eczema (Koyama et al., 2010; Kukkonen et al., 2007; Taylor, Dunstan, & Prescott, 2007), and their use in clinical practice is still not officially encouraged (Fiocchi et al., 2012). Therefore, although some data are promising thus far (Isolauri, Rautava, & Salminen, 2012), more research is still needed to demonstrate strain-specific benefits in the prevention of a range of allergic diseases (Prescott & Nowak-Wegrzyn, 2011; Toh et al., 2012).

1.5.3 Urogenital infections

The human vagina is also colonised by over 250 different species of bacteria comprising the vaginal microbiota, and it is dominated particularly by *Lactobacillus* species. Similarly to the colonic microbiota, dysbiosis in the vaginal microbiota has been linked with diseases such as bacterial vaginosis and aerobic vaginitis, where increased levels of pathogenic bacteria in the microbial community cause inflammation (Li, McCormick, Bocking, & Reid, 2012). Probiotic treatment with different *Lactobacillus* species holds much promise in the treatment of urogenital infections. This may also have even more long-lasting effects besides reduced risk of infections, since the vaginal microbiota is associated with conception and pregnancy and it is the first microbial community that the newborn faces during labour (Reid, Bisanz, Monachese, Burton, & Reid, 2013). However, to date very few studies have explored probiotic effects in this area of human health.

1.5.4 Rheumatic diseases

Evidence exists that dysbiosis of colonic microbiota is also contributing to initiation and perpetuation of inflammatory joint diseases, such as rheumatoid arthritis (RA) and spondyloarthritis. Theories of the mechanisms through which the microbiota may influence the pathogenesis of these diseases include increased permeability of the gastrointestinal epithelia, loss of tolerance towards indigenous microbiota members, and trafficking of activated proinflammatory immune cells and cytokines and antigenic material between the gastrointestinal tract and joints (Yeoh, Burton, Suppiah, Reid, & Stebbings, 2013). The potential of probiotics to influence the inflammatory processes in these diseases has been investigated to some extent in animals and to a very limited extent in humans. Two human studies on RA patients have not been able to demonstrate any benefits attributable to probiotic use on the clinical symptoms of the disease, but the reported quality of life (Pineda Mde et al., 2011) and wellbeing of the RA patients (Hatakka et al., 2003) were improved during probiotic therapy. Probiotic supplementation did not interfere with the medical therapy of RA, when probiotics were used simultaneously with sulfasalazine therapy (Lee et al., 2010).

1.6 Metabolic health

In addition to the evidence on the impact on human immunity, evidence has accumulated during the past decade on the capability of the intestinal microbiota to participate in and also influence the metabolism of its human host. The effects of probiotics are still little studied in this area, but considering the global epidemic of obesity and metabolic diseases, this application holds huge possibilities if probiotic efficacy can be scientifically demonstrated and the epidemic of "Western diseases" ameliorated.

1.6.1 Obesity

Obesity is a metabolic disorder that is impacting humans at an increasing pace all over the world, and it predisposes people to diseases such as increased blood pressure, atherosclerosis, metabolic syndrome and diabetes. The role of intestinal microbiota composition in obesity began to be uncovered when an altered ratio of two dominant colonic microbial phyla, *Bacteroidetes* and *Firmicutes*, was demonstrated between lean and obese rodents (Bäckhed et al., 2004; Ley et al., 2005) and humans (Turnbaugh et al., 2009). Furthermore, decreased levels of bifidobacteria and increased levels of *S. aureus* in infant faeces have been shown to precede increased adiposity of children at 7 years of age (Kalliomäki et al., 2008). The obese microbiome has an increased capacity to harvest energy from the diet and it impacts the deposition of fat into adipose tissue (Bäckhed, Manchester, Semenkovich, & Gordon, 2007; Samuel et al., 2008). On the other hand, the diet that humans typically consume influences the microbiota structure, and thus the role of microbes in energy balance together with dietary factors and genotype all seem to contribute to increased adiposity and development of obesity (Sanz, Rastmanesh, & Agostonic, 2013). Owing to the partial contribution of colonic microbes to the obesity epidemic, intervention trials have investigated the possibility of probiotics to impact adiposity. Few studies have demonstrated a beneficial effect of probiotic microbes on adiposity: in an intervention trial with *Lactobacillus gasseri* SBT2055, the consumption of fermented milk with this probiotic for 3 months was shown to significantly reduce abdominal visceral and subcutaneous fat, weight and body mass index (BMI) of subjects with elevated BMI at the beginning of the trial (Kadooka et al., 2010). In another intervention with pregnant women, probiotic *L. rhamnosus* GG, together with *B. animalis* subsp. *lactis* Bb-12 and dietary counselling, was shown to reduce central adiposity in the postpartum period (Ilmonen, Isolauri, Poussa, & Laitinen, 2011). In a 10-year follow-up study on children, pre- and postnatal consumption of *L. rhamnosus* GG inhibited excessive weight gain in children (Luoto, Kalliomaki, Laitinen, & Isolauri, 2010). It goes without saying that more research is needed in this area before definite answers on the protective mechanisms of probiotics against obesity can be given (Sanz et al., 2013).

1.6.2 Metabolic syndrome and diabetes

In the same study in which *L. rhamnosus* GG and *B. animalis* subsp. *lactis* Bb-12 were shown to impact central adiposity, the probiotic intervention also improved glucose tolerance and insulin sensitivity during pregnancy and 12 months postpartum

(Laitinen, Poussa, Isolauri, Nutrition, & Mucosal Immunology and Intestinal Microbiota Group, 2009). Furthermore, the risk of the mothers to develop gestational diabetes was decreased in the probiotic groups (Luoto, Laitinen, Nermes, & Isolauri, 2010). Thus, in addition to impacting the energy balance of humans, gut microbiota and probiotics seem to participate in the regulation of glucose and insulin metabolism and impact the risk of developing metabolic diseases.

Several other studies have investigated the link between colonic microbes, probiotics and onset of diabetes, although mainly in animal models. An inverse correlation between the numbers of colonic bifidobacteria and serum concentrations of lipopolysaccharide (LPS), a component of the bacterial cell wall, has been reported in mice fed a high-fat diet (Cani et al., 2007). These studies have led to the hypothesis of metabolic endotoxaemia, where a high-fat diet causes dysbiosis in the colonic microbiota composition and leads to increased permeability of the intestine, further contributing to increased concentrations of LPS and systemic low-grade inflammation. Chronic inflammation impairs the function of several organs and contributes to the development of metabolic syndrome and diabetes (Cani et al., 2008). These results have been, to some extent, seen also in humans, since endotoxaemia (i.e. increased LPS in blood) was significantly associated with increased risk for prevalent and incident diabetes, and the LPS activity was linearly related to several components of metabolic syndrome (Pussinen, Havulinna, Lehto, Sundvall, & Salomaa, 2011). Nevertheless, although the link between microbiota modulation, metabolic health, and development of metabolic syndrome and diabetes has been demonstrated, the proof of beneficial effects deriving from probiotics is still mostly lacking. In animals, the antidiabetic effects of *B. animalis* subsp. *lactis* 420 (Amar et al., 2011) and probiotic dahi containing *L. acidophilus* and *L. casei* (Yadav, Jain, & Sinha, 2007) have been demonstrated, but in humans the results have been inconsistent; neither *L. casei* Shirota (Leber et al., 2012) nor *L. salivarius* Ls-33 (Gobel, Larsen, Jakobsen, Molgaard, & Michaelsen, 2012) was able to influence the parameters of metabolic syndrome or inflammatory markers in adolescents and adults.

1.6.3 Hypercholesterolaemia

Elevated serum cholesterol level is an acknowledged risk factor for atherosclerosis and coronary heart disease, which are still among the leading causes of mortality in the world. Lactic acid bacteria have been recognised as potential mediators that would have a beneficial impact on hypercholesterolaemia; lactobacilli possess enzymes that participate in the deconjugation of bile salts to form primary bile acids, thereby inhibiting micelle formation, cholesterol absorption from the intestine and the enterohepatic circulation of cholesterol. Also, systemic effects are thought to derive from the metabolic activity of probiotic microbes and increased concentrations of fatty acids (acetic, propionic and butyric acids), which may impact the cholesterol synthesis and lipid metabolism systemically (Aggarwal, Swami, & Kumar, 2013). Animal studies have shown quite consistent cholesterol-lowering effects of various probiotic microbes (Huang, Wang, et al., 2013; Huang, Wu, et al., 2013), but the data from human studies are very inconsistent (Aggarwal et al., 2013).

1.7 Summary

Probiotics in fermented foods have the potential to modify the composition of the intestinal microbiota, which has been shown to be altered in several diseases of the human host. *Lactobacillus* and *Bifidobacterium* are the two probiotic microbial genera that have been investigated by far the most, perhaps because of their dominance in the traditionally used fermented foods and (except for *Bifidobacterium dentium*) the absence of typical pathogenic microbes within these genera. Through restoring the intestinal microbiota balance and impacting on the function of the immune system, probiotics may be effective in preventing the development of chronic immune-mediated diseases such as metabolic, inflammatory and bowel diseases. Strong scientific evidence suggests that different probiotic microbes may reduce infants' risk of developing atopic diseases or necrotizing colitis and may protect children and adults against antibiotic-associated diarrhoea and upper respiratory tract infections. There are also implications that probiotics may play a role in reducing the risk of gastrointestinal inflammatory diseases and may also regulate the metabolism of humans. Progress in understanding the mechanisms by which the colonic microbiota impact other mucosal surfaces of the body and the function of the immune system has shown that the influence of probiotic microbes reaches way beyond the gastrointestinal tract. Nevertheless, much more research is needed to confirm these benefits in humans. It is also important to bear in mind that the demonstrated health effects are strain-specific and cannot be extrapolated to other probiotic microbes.

1.8 Future trends

New applications for probiotic delivery will certainly be found. However, it is also likely that the current mainstream vehicles for probiotic consumption, i.e. yogurt-type dairy products and supplements (capsules), will remain the leading carriers of probiotics in the near future. This is not so much a technological issue, given the options of other possible carriers that are available, as discussed. It is, however, a matter of consumer acceptance.

New strains will likely be introduced into the market. In addition to strains from the genera *Lactobacillus* and *Bifidobacterium*, strains from other genera, such as *Propionibacterium* and *Lactococcus*, commonly found in fermented foods, are likely to receive more attention. But strains from "new" probiotic genera are also likely to emerge, such as butyrate-producing *Roseburia* and *Clostridium*, or strains from the anti-inflammatory species *F. prausnitzii*.

Furthermore, genetically modified probiotics should be anticipated. Although genetically modified organisms meet resistance in certain parts of the world, they will probably be accepted eventually, provided that they have clear benefits to the user, and they may even target diseases for which there is currently no cure. An example of this is the interleukin-10-producing *Lactococcus lactis* for treatment of Crohn disease (Braat et al., 2006). In the more distant future, individualised strains and combinations

of strains may become available; this may depend on one's own microbiota (Wu et al., 2011) or blood group (Mäkivuokko et al., 2012).

Existing health targets, as described above, will be further investigated and either confirmed or refuted for probiotic efficacy. New targets will also certainly emerge. Many of these targets will be indicated by the substantial research efforts towards understanding of the intestinal and other microbiota. The influence of the intestinal microbiota on, for instance, the gut–brain axis may hint at possibilities for probiotics and stress or mental awareness.

To what extent strains from such new probiotic species can and will be incorporated into fermented foods is uncertain. Likewise, it is uncertain whether these and other new health targets are suitable for fermented foods. What is certain, however, is that the "one-strain-suits-all" approach that has been common until now is going to change. A single strain is simply not able to perform the varied functions that are required to tackle various health risks; the emergence of health benefit-specific strains should be anticipated. Similarly, one could anticipate that more complex mixtures might be more efficacious, as they could provide a multitude of functions to microbiota in disarray.

1.9 Sources of further information and advice

- Lahtinen, S., Ouwehand, A. C., Salminen, S., & von Wright, A. (Eds.). (2012). *Lactic acid bacteria, microbiological and functional aspects* (4th ed.). Boca Raton, FL: CRC Press, Taylor & Francis Group.
  Reference book on the lactic acid bacteria and bifidobacteria; health, technological and regulatory aspects.
- Binns, N. (2013). Probiotics, prebiotics and the gut microbiota, ILSI Europe.
  Reference monograph especially for the interested lay person; download for free from: http://www.ilsi.org/Europe/Pages/ConciseMonographSeries.aspx.
- Fredricks, D. N. (Ed.). (2013) *The human microbiota: How microbial communities affect health and disease* (1st ed.). Hoboken, NJ: John Wiley & Sons, Inc.
  Reference book describing the microbiota occupying different parts of the human body.
- ISAPP (International Scientific Association for Probiotics and Prebiotics) webpages: www.isapp.net.
  Up-to-date information on probiotics and human microbiota from an international nonprofit organisation of scientists.
- International Probiotics Association (IPA) webpages: http://internationalprobiotics.org/home/.
  Platform of probiotic-producing industries.

References

Aggarwal, J., Swami, G., & Kumar, M. (2013). Probiotics and their effects on metabolic diseases: an update. *Journal of Clinical and Diagnostic Research*, *7*(1), 173–177.

Ahanchian, H., Jones, C. M., Chen, Y. S., & Sly, P. D. (2012). Respiratory viral infections in children with asthma: do they matter and can we prevent them? *BMC Pediatrics*, *12*, 147. http://dx.doi.org/10.1186/1471-2431-12-147.

Amar, J., Chabo, C., Waget, A., Klopp, P., Vachoux, C., Bermudez-Humaran, L. G., et al. (2011). Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. *EMBO Molecular Medicine*, *3*(9), 559–572.

Avadhani, A., & Miley, H. (2011). Probiotics for prevention of antibiotic-associated diarrhea and *Clostridium difficile*-associated disease in hospitalized adults–a meta-analysis. *Journal of the American Academy of Nurse Practitioners*, *23*, 269–274.

Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(44), 15718–15723.

Bäckhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(3), 979–984.

Bisgaard, H., Li, N., Bonnelykke, K., Chawes, B. L., Skov, T., Paludan-Muller, G., et al. (2011). Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *The Journal of Allergy and Clinical Immunology*, *128*(3), 646–652.e1–5.

Braat, H., Rottiers, P., Hommes, D. W., Huyghebaert, N., Remaut, E., Remon, J. P., et al. (2006). A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clinical Gastroenterology and Hepatology*, *4*, 754–759.

Braegger, C., Chmielewska, A., Decsi, T., Kolacek, S., Mihatsch, W., Moreno, L., et al. (2011). Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, *52*, 238–250.

Brenner, D. M., Moeller, M. J., Chey, W. D., & Schoenfeld, P. S. (2009). The utility of probiotics in the treatment of irritable bowel syndrome: a systematic review. *The American Journal of Gastroenterology*, *104*, 1033–1050.

Canani, R. B., & Di Costanzo, M. (2013). Gut microbiota as potential therapeutic target for the treatment of cow's milk allergy. *Nutrients*, *5*(3), 651–662.

Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., et al. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*, *57*(6), 1470–1481.

Cani, P. D., Neyrinck, A. M., Fava, F., Knauf, C., Burcelin, R. G., Tuohy, K. M., et al. (2007). Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia*, *50*(11), 2374–2383.

Clarke, G., Cryan, J. F., Dinan, T. G., & Quigley, E. M. (2012). Review article: probiotics for the treatment of irritable bowel syndrome–focus on lactic acid bacteria. *Alimentary Pharmacology and Therapeutics*, *35*, 403–413.

Cox, M. J., Huang, Y. J., Fujimura, K. E., Liu, J. T., McKean, M., Boushey, H. A., et al. (2010). *Lactobacillus casei* abundance is associated with profound shifts in the infant gut microbiome. *PLoS One*, *5*(1), e8745.

Davidson, R. H., Duncan, S. E., Hackney, C. R., Eigel, W. N., & Boling, J. W. (2000). Probiotic culture survival and implications in fermented frozen yogurt characteristics. *Journal of Dairy Science*, *83*, 666–673.

Doege, K., Grajecki, D., Zyriax, B. C., Detinkina, E., Zu Eulenburg, C., & Buhling, K. J. (2012). Impact of maternal supplementation with probiotics during pregnancy on atopic eczema in childhood–a meta-analysis. *The British Journal of Nutrition*, *107*(1), 1–6.

Engelbrektson, A., Korzenik, J. R., Pittler, A., Sanders, M. E., Klaenhammer, T. R., Leyer, G., et al. (2009). Probiotics to minimize the disruption of faecal microbiota in healthy subjects undergoing antibiotic therapy. *Journal of Medical Microbiology*, *58*, 663–670.

FAO/WHO. (2002). *Guidelines for the evaluation of probiotics in food*. pp. 1–11. http://www.who.int/foodsafety/publications/fs_management/probiotics2/en/.

Fiocchi, A., Burks, W., Bahna, S. L., Bielory, L., Boyle, R. J., Cocco, R., et al. (2012). Clinical use of probiotics in pediatric allergy (CUPPA): a world allergy organization position paper. *The World Allergy Organization Journal*, *5*(11), 148–167.

Forssten, S. D., Sindelar, C. W., & Ouwehand, A. C. (2011). Probiotics from an industrial perspective. *Anaerobe*, *17*, 410–413.

Fujimura, K. E., Slusher, N. A., Cabana, M. D., & Lynch, S. V. (2010). Role of the gut microbiota in defining human health. *Expert Review of Anti-infective Therapy*, *8*(4), 435–454.

Gleeson, M., Bishop, N. C., Oliveira, M., & Tauler, P. (2011). Daily probiotic's (*Lactobacillus casei* Shirota) reduction of infection incidence in athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, *21*(1), 55–64.

Gobel, R. J., Larsen, N., Jakobsen, M., Molgaard, C., & Michaelsen, K. F. (2012). Probiotics to adolescents with obesity: effects on inflammation and metabolic syndrome. *Journal of Pediatric Gastroenterology and Nutrition*, *55*(6), 673–678.

Hao, Q., Lu, Z., Dong, B. R., Huang, C. Q., & Wu, T. (2011). Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database of Systematic Reviews (Online)* (9), CD006895. http://dx.doi.org/10.1002/14651858.CD006895.pub2.

Hatakka, K., Martio, J., Korpela, M., Herranen, M., Poussa, T., Laasanen, T., et al. (2003). Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis–a pilot study. *Scandinavian Journal of Rheumatology*, *32*(4), 211–215.

Hedin, C., Whelan, K., & Lindsay, J. O. (2007). Evidence for the use of probiotics and prebiotics in inflammatory bowel disease: a review of clinical trials. *The Proceedings of the Nutrition Society*, *66*, 307–315.

Hempel, S., Newberry, S. J., Maher, A. R., Wang, Z., Miles, J. N. V., Shanman, R., et al. (2012). Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *The Journal of the American Medical Association*, *307*, 1959–1969.

Holzapfel, W. H., & Schillinger, U. (2002). Introduction to pre- and probiotics. *Food Research International*, *35*, 109–116.

Huang, Y., Wang, X., Wang, J., Wu, F., Sui, Y., Yang, L., et al. (2013). *Lactobacillus plantarum* strains as potential probiotic cultures with cholesterol-lowering activity. *Journal of Dairy Science*, *95*(5), 2746–2753. http://dx.doi.org/10.3168/jds.2012-6123.

Huang, Y., Wu, F., Wang, X., Sui, Y., Yang, L., & Wang, J. (2013). Characterization of *Lactobacillus plantarum* Lp27 isolated from Tibetan kefir grains: a potential probiotic bacterium with cholesterol-lowering effects. *Journal of Dairy Science*, *96*(5), 2816–2825. http://dx.doi.org/10.3168/jds.2012-6371.

Ibrahim, F., & Ouwehand, A. C. (2011). *Potential of probiotics in reducing intestinal transit time*. Touchgastroenterology.com.

Ibrahim, F., Ruvio, S., Granlund, L., Salminen, S., Viitanen, M., & Ouwehand, A. C. (2010). Probiotics and immunosenescence: cheese as a carrier. *FEMS Immunology and Medical Microbiology*, *59*, 53–59.

Ilmonen, J., Isolauri, E., Poussa, T., & Laitinen, K. (2011). Impact of dietary counselling and probiotic intervention on maternal anthropometric measurements during and after pregnancy: a randomized placebo-controlled trial. *Clinical Nutrition (Edinburgh, Scotland)*, *30*(2), 156–164.

Isolauri, E., Rautava, S., & Salminen, S. (2012). Probiotics in the development and treatment of allergic disease. *Gastroenterology Clinics of North America*, *41*(4), 747–762.

Jung, J. Y., Lee, S. H., Kim, J. M., Park, M. S., Bae, J. W., Hahn, Y., et al. (2011). Metagenomic analysis of kimchi, a traditional Korean fermented food. *Applied and Environmental Microbiology*, *77*, 2264–2274.

Kadooka, Y., Sato, M., Imaizumi, K., Ogawa, A., Ikuyama, K., Akai, Y., et al. (2010). Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. *European Journal of Clinical Nutrition*, *64*(6), 636–643.

Kalliomaki, M., Collado, M. C., Salminen, S., & Isolauri, E. (2008). Early differences in fecal microbiota composition in children may predict overweight. *The American Journal of Clinical Nutrition*, *87*(3), 534–538.

Kalliomaki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P., & Isolauri, E. (2001). Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet*, *357*(9262), 1076–1079.

Kalliomäki, M., Salminen, S., Poussa, T., & Isolauri, E. (2007). Probiotics during the first 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. *The Journal of Allergy and Clinical Immunology*, *119*(4), 1019–1021.

Kang, E. J., Kim, S. Y., Hwang, I. H., & Ji, Y. J. (2013). The effect of probiotics on prevention of common cold: a meta-analysis of randomized controlled trial studies. *Korean Journal of Family Medicine*, *34*(1), 2–10.

Kelly, C. J., Colgan, S. P., & Frank, D. N. (2012). Of microbes and meals: the health consequences of dietary endotoxemia. *Nutrition in Clinical Practice*, *27*, 215–225.

Koyama, T., Kirjavainen, P. V., Fisher, C., Anukam, K., Summers, K., Hekmat, S., et al. (2010). Development and pilot evaluation of a novel probiotic mixture for the management of seasonal allergic rhinitis. *Canadian Journal of Microbiology*, *56*(9), 730–738.

Kukkonen, K., Savilahti, E., Haahtela, T., Juntunen-Backman, K., Korpela, R., Poussa, T., et al. (2007). Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *The Journal of Allergy and Clinical Immunology*, *119*(1), 192–198.

Laitinen, K., Poussa, T., Isolauri, E., & Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota Group. (2009). Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. *The British Journal of Nutrition*, *101*(11), 1679–1687.

Leber, B., Tripolt, N. J., Blattl, D., Eder, M., Wascher, T. C., Pieber, T. R., et al. (2012). The influence of probiotic supplementation on gut permeability in patients with metabolic syndrome: an open label, randomized pilot study. *European Journal of Clinical Nutrition*, *66*(10), 1110–1115.

Lee, H. J., Waller, R. D., Stebbings, S., Highton, J., Orlovich, D. A., Schmierer, D., et al. (2010). The effects of an orally administered probiotic on sulfasalazine metabolism in individuals with rheumatoid arthritis: a preliminary study. *International Journal of Rheumatic Diseases*, *13*(1), 48–54.

Lewis, S. J., & Heaton, K. W. (1997). Stool form scale as a useful guide to intestinal transit time. *Scandinavian Journal of Gastroenterology*, *32*, 920–924.

Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(31), 11070–11075.

Leyer, G. J., Li, S., Mubasher, M. E., Reifer, C., & Ouwehand, A. C. (2009). Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics*, *124*(2), e172–e179.

Li, J., McCormick, J., Bocking, A., & Reid, G. (2012). Importance of vaginal microbes in reproductive health. *Reproductive Sciences (Thousand Oaks, Calif.)*, *19*(3), 235–242.

Luoto, R., Kalliomaki, M., Laitinen, K., & Isolauri, E. (2010). The impact of perinatal probiotic intervention on the development of overweight and obesity: follow-up study from birth to 10 years. *International Journal of Obesity (2005)*, *34*(10), 1531–1537.

Luoto, R., Laitinen, K., Nermes, M., & Isolauri, E. (2010). Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. *The British Journal of Nutrition*, *103*(12), 1792–1799.

Mäkivuokko, H., Lahtinen, S. J., Wacklin, P., Tuovinen, E., Tenkanen, H., Nikkila, J., et al. (2012). Association between the ABO blood group and the human intestinal microbiota composition. *BMC Microbiology*, *12*, 94.

Mcdonough, F. E., Hitchins, A. D., Wong, N. P., Wells, P., & Bodwell, C. E. (1987). Modification of sweet acidophilus milk to improve utilization by lactose-intolerant persons. *American Journal of Clinical Nutrition*, *45*, 570–574.

Mcfarland, L. V. (2007). Meta-analysis of probiotics for the prevention of traveler's diarrhea. *Travel Medicine and Infectious Disease*, *5*, 97–105.

Metchnikoff, I. I. (1907). *The prolongation of life. Optimistic studies*. London: Heinemann.

Mimura, T., Rizzello, F., Helwig, U., Poggioli, G., Schreiber, S., Talbot, I. C., et al. (2004). Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut*, *53*, 108–114.

Molin, G. (2001). Probiotics in foods not containing milk or milk components, with special reference to *Lactobacillus plantarum* 299v. *American Journal of Clinical Nutrition*, *73*, 380S–385S.

Nout, M. J. (2009). Rich nutrition from the poorest – cereal fermentations in Africa and Asia. *Food Microbiology*, *26*, 685–692.

Noval Rivas, M., Burton, O. T., Wise, P., Zhang, Y. Q., Hobson, S. A., Garcia Lloret, M., et al. (2013). A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *The Journal of Allergy and Clinical Immunology*, *131*(1), 201–212.

Nylund, L., Satokari, R., Nikkilä, J., Rajilic-Stojanovic, M., Kalliomäki, M., Isolauri, E., et al. (2013). Microarray analysis reveals marked intestinal microbiota aberrancy in infants having eczema compared to healthy children in at-risk for atopic disease. *BMC Microbiology*, *13*, 12. http://dx.doi.org/10.1186/1471-2180-13-12.

Odamaki, T., Xiao, J. Z., Iwabuchi, N., Sakamoto, M., Takahashi, N., Kondo, S., et al. (2007). Fluctuation of fecal microbiota in individuals with Japanese cedar pollinosis during the pollen season and influence of probiotic intake. *Journal of Investigational Allergology and Clinical Immunology: Official Organ of the International Association of Asthmology (INTERASMA) and Sociedad Latinoamericana de Alergia e Inmunologia*, *17*(2), 92–100.

Ouwehand, A. C., Nermes, M., Collado, M. C., Rautonen, N., Salminen, S., & Isolauri, E. (2009). Specific probiotics alleviate allergic rhinitis during the birch pollen season. *World Journal of Gastroenterology*, *15*(26), 3261–3268.

Pelucchi, C., Chatenoud, L., Turati, F., Galeone, C., Moja, L., Bach, J. F., et al. (2012). Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. *Epidemiology (Cambridge, Mass.)*, *23*(3), 402–414.

Pineda Mde, L., Thompson, S. F., Summers, K., de Leon, F., Pope, J., & Reid, G. (2011). A randomized, double-blinded, placebo-controlled pilot study of probiotics in active rheumatoid arthritis. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, *17*(6), CR347–54.

Prescott, S., & Nowak-Wegrzyn, A. (2011). Strategies to prevent or reduce allergic disease. *Annals of Nutrition and Metabolism*, *59*(Suppl. 1), 28–42.

Purchiaroni, F., Tortora, A., Gabrielli, M., Bertucci, F., Gigante, G., Ianiro, G., et al. (2013). The role of intestinal microbiota and the immune system. *European Review for Medical and Pharmacological Sciences*, *17*(3), 323–333.

Pussinen, P. J., Havulinna, A. S., Lehto, M., Sundvall, J., & Salomaa, V. (2011). Endotoxemia is associated with an increased risk of incident diabetes. *Diabetes Care*, *34*(2), 392–397.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*, 59–65.

Reid, J. N., Bisanz, J. E., Monachese, M., Burton, J. P., & Reid, G. (2013). The Rationale for probiotics improving reproductive health and pregnancy outcome. *American Journal of Reproductive Immunology*, *69*(5), 558–566. http://dx.doi.org/10.1111/aji.12086.

Rekha, C. R., & Vijayalakshmi, G. (2010). Bioconversion of isoflavone glycosides to aglycones, mineral bioavailability and vitamin B complex in fermented soymilk by probiotic bacteria and yeast. *Journal of Applied Microbiology*, *109*, 1198–1208.

Rerksuppaphol, S., & Rerksuppaphol, L. (2012). Randomized controlled trial of probiotics to reduce common cold in schoolchildren. *Pediatrics International: Official Journal of the Japan Pediatric Society*, *54*(5), 682–687.

Rey, E., & Talley, N. J. (2009). Irritable bowel syndrome: novel views on the epidemiology and potential risk factors. *Digestive and Liver Disease: Official Journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver*, *41*, 772–780.

Ritchie, M. L., & Romanuk, T. N. (2012). A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLoS One*, *7*. e34938 (11pp.).

Rousseaux, C., Thuru, X., Gelot, A., Barnich, N., Neut, C., Dubuquoy, L., et al. (2007). *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. *Nature Medicine*, *13*, 35–37.

Samuel, B. S., Shaito, A., Motoike, T., Rey, F. E., Backhed, F., Manchester, J. K., et al. (2008). Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(43), 16767–16772.

Sanz, Y., Rastmanesh, R., & Agostonic, C. (2013). Understanding the role of gut microbes and probiotics in obesity: how far are we? *Pharmacological Research: The Official Journal of the Italian Pharmacological Society*, *69*(1), 144–155.

Singh, A., Hacini-Rachinel, F., Gosoniu, M. L., Bourdeau, T., Holvoet, S., Doucet-Ladeveze, R., et al. (2013). Immune-modulatory effect of probiotic *Bifidobacterium lactis* NCC2818 in individuals suffering from seasonal allergic rhinitis to grass pollen: an exploratory, randomized, placebo-controlled clinical trial. *European Journal of Clinical Nutrition*, *67*(2), 161–167.

Smith, T. J., Rigassio-Radler, D., Denmark, R., Haley, T., & Touger-Decker, R. (2013). Effect of *Lactobacillus rhamnosus* LGG(R) and *Bifidobacterium animalis* ssp. *lactis* BB-12(R) on health-related quality of life in college students affected by upper respiratory infections. *British Journal of Nutrition*, *109*(11), 1999–2007. http://dx.doi.org/10.1017/S0007114512004138.

Sokol, H., Seksik, P., Furet, J. P., Firmesse, O., Nion-Larmurier, I., Beaugerie, L., et al. (2009). Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflammatory Bowel Diseases*, *15*, 1183–1189.

Taylor, A. L., Dunstan, J. A., & Prescott, S. L. (2007). Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *The Journal of Allergy and Clinical Immunology*, *119*(1), 184–191.

Toh, Z. Q., Anzela, A., Tang, M. L. K., & Licciardi, P. V. (2012). Probiotic therapy as a novel approach for allergic disease. *Frontiers in Pharmacology*, *3*(171). 0.3389/fphar.2012.00171.

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., et al. (2009). A core gut microbiome in obese and lean twins. *Nature*, *457*(7228), 480–484.

Van Niel, C. W., Feudtner, C., Garrison, M. M., & Christakis, D. A. (2002). Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics*, *109*, 678–684.

Vriens, H. P. (2009). Restoring the balance: why chocolate proves to be an ideal carrier for probiotics. *NutraCos*, *3*, 6–7.

Waller, P. A., Gopal, P. K., Leyer, G. J., Ouwehand, A. C., Reifer, C., Stewart, M. E., et al. (2011). Dose-response effect of *Bifidobacterium lactis* HN019 on whole gut transit time and functional gastrointestinal symptoms in adults. *Scandinavian Journal of Gastroenterology*, *46*, 1057–1064.

Wang, Q., Dong, J., & Zhu, Y. (2012). Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low-birth-weight infants: an updated meta-analysis of 20 randomized, controlled trials. *Journal of Pediatric Surgery*, *47*, 241–248.

Wang, Z.-H., Gao, Q.-Y., & Fang, J.-Y. (2013). Meta-analysis of the efficacy and safety of Lactobacillus-containing and Bifidobacterium-containing probiotic compound preparation in *Helicobacter pylori* eradication therapy. *Journal of Clinical Gastroenterology*, *47*, 25–32.

Wickens, K., Black, P., Stanley, T. V., Mitchell, E., Barthow, C., Fitzharris, P., et al. (2012). A protective effect of *Lactobacillus rhamnosus* HN001 against eczema in the first 2 years of life persists to age 4 years. *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology*, *42*(7), 1071–1079.

Wickens, K., Black, P. N., Stanley, T. V., Mitchell, E., Fitzharris, P., Tannock, G. W., et al. (2008). A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. *The Journal of Allergy and Clinical Immunology*, *122*(4), 788–794.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, *334*, 105–108.

Yadav, H., Jain, S., & Sinha, P. R. (2007). Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats. *Nutrition (Burbank, Los Angeles County, Calif.)*, *23*(1), 62–68.

Yeoh, N., Burton, J. P., Suppiah, P., Reid, G., & Stebbings, S. (2013). The role of the microbiome in rheumatic diseases. *Current Rheumatology Reports*, *15*(3), 314. http://dx.doi.org/10.1007/s11926-012-0314-y.